Fingermarks, more than just a ridge pattern

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APPENDIX 2

SUPPORTING INFORMATION FOR CHAPTER 9:

ON THE AUTOFLUORESCENCE OF AGED FINGERMARKS

This section contains three tables with the Rf-values of the reference compounds studied with TLC. In addition, more detailed information is given about the reference compounds that did not match on all four criteria with the fluorescent spots obtained from the fingermarks: 1) the color of the fluorescent spot, which was determined by visual examination; and categorized in a purple, blue, green, yellow or white color; 2) the Rf-value of the fluorescent compounds; 3) fluorescent excitation and emission spectra of the spot; 4) color reaction with Ehrlich's reagent. However, it was not possible to obtain excitation and emission spectra from all fluorescent spots observed on the developed TLC-plates, because of the low fluorescent intensity of the spots.

Stock solutions were prepared in 1% methanol and 2µl was spotted on the TLC plates.

Table S1. Rf-values of reference compounds (n≥3), t: spot is tailed

Compound	Chloroform/methanol (1:4)			
	mg/ml	Rf-value (sd)	Color spot	
			UV	Blue
Aminoacetophenone	10	0.36 (0.01) 0.94 (0.01)	Blue Blue	Yellow
Anthranilic acid	1	0.94 (0.01)	Blue	
Formylkynurenine	1	0.46 (0.03)	Blue	
3-hydroxyanthranilic acid	1	0.97 (0.01)	Blue	
3-hydroxykynurenine	1	0.48 (0.03) t	Yellow	Yellow
8-hydroxy quinaldic acid	1	0.91 (0.01)	Yellow	Yellow
Harman	1	0.79 (0.02)	Blue	
Indole acetic acid	10	0.98 (0.01)	Blue	Yellow
Kynurenic acid	10	0,80 (0,015) 0.93 (0.004) 0.98 (0.003)	White Purple	 Yellow Yellow
Kynurenine	10	0,56 (0.01) t 0.93 (0.02)	Blue Yellow	
Norharman	1	0.84 (0.02)	Blue	
Tryptophan	10	0.67 (0.01) t	Blue	
Xanthurenic acid	1	0.93 (0.02) 0.98 (0.01)	Yellow Yellow	Green

Table S2. Rf-values of reference compound, aged tryptophan stored under office conditions and stored in a dark room (n≥6)

Compound	Chloroform/methanol (1:4)			
	mg/ml	Rf-value (sd)	Color spot	
			UV	Blue
Tryptophan (3 weeks old) office conditions	10	0.21 (0.01) t	Yellow	
		0.37 (0.01)	Blue	
		0.74 (0.01)	Blue	
		0.88 (0.02)	Blue	Yellow
		0.94 (0.004)	Blue	Yellow
Tryptophan (2 weeks old) office conditions	10	0.21 (0.01) t	Yellow	
		0.38 (0.08)	Yellow	
		0.72 (0.03)	Blue	
		0.82 (0.03)	Blue	Yellow
		0.89 (0.01)	Purple	
		0.93 (0.004)	Blue	Yellow
Tryptophan (1 week old) office conditions	10	0.30 (0.08)t	Yellow	
		0.47 (0.09)t	Yellow	
		0.73 (0.02)	Blue	
		0.84 (0.01)	Blue	Yellow
		0.89 (0.008)	Purple	
		0.91 (0.007)	Purple	
		0.95 (0.006)	Yellow	Yellow
Tryptophan (3 weeks old) dark room	10	0.07 (0.007) t	Yellow	Yellow
		0.20 (0.05) t	Yellow	
		0.336 (0.02)	Yellow	
		0.84 (0.007)	Blue	Yellow
		0.90 (0.007)	Purple	
		0.94 (0.006)	Yellow	Yellow
Tryptophan (2 weeks old) dark room	10	0.23 (0.07)t	Yellow	
		0.37 (0.02)		Yellow
		0.72 (0.002)	Blue	
		0.83 (0.01)	Blue	Yellow
		0.91 (0.005)	Purple	
		0.95 (0.003)	Blue	Yellow
Tryptophan (1 week old) dark room	10	0.37 (0.08)t	Blue	
		0.85 (0.02)	Blue	
		0.87 (0.02)	Blue	Yellow
		0.89 (0.004)	Yellow	Yellow
		0.92 (0.02)	Purple	
		0.96 (0.002)	Blue	Yellow

Table S3. Rf-values of reference compound, aged indoleacetic acid stored under office conditions and stored in a dark room (n=1)

Compound	chloroform/methanol (1:4)			
	mg/ml	Rf-value	Color spot	
			UV	Blue
Indoleacetic acid (3 weeks old) office conditions	10	0.84 0.88 0.96	Yellow Yellow	Yellow Yellow Yellow
Indoleacetic acid (2 weeks old) office conditions	10	0.85 0.95	Yellow Yellow	Yellow Yellow
Indoleacetic acid (1 week old) office conditions	10	0.84 0.92 0.97	White Yellow	Yellow Yellow
Indole acetic acid (3weeks old) dark room	10	0.83 0.97	Yellow Yellow	Yellow Yellow
Indoleacetic acid (2 weeks old) dark room	10	0.84 0.94	Yellow Yellow	Yellow
Indoleacetic acid (1 week old) dark room	10	0.85 0.95	Yellow Yellow	Yellow Yellow

RESULTS REFERENCE COMPOUNDS

Aminoacetophenone displays a strong pale blue fluorescent signal [1]. After development, two separate fluorescent spots can be observed, one purple spot (Rf-value 0.36) and a blue spot (Rf-value 0.94). None of the spots can be excluded of contributing to the fluorescence of aged fingermarks. The Rf-value of 0.36 is similar to spot A found in fingermarks stored in a dark room. However, the fluorescent color of this spot is different than the one observed in spot A, namely blue instead of purple. The blue spot with an Rf-value (0.94) shows similarities with spot F/G (light/dark). No color reaction was observed of aminoacetophenone with Ehrlich's reagent. No match was found between the excitation and emission spectra of aminoacetophenone and the obtained spectra from the eluted fingermarks. Based on our findings, aminoacetophenone was excluded as a fluorescent fingermark component.

Anthranilic acid is a blue fluorescent metabolite of tryptophan [1-2]. Anthranilic acid eluted into one fluorescent spot with an Rf-value 0.94. Based on the color of the fluorescent spot and the Rf-value, anthranilic acid from fingermarks stored under office conditions and dark could not be excluded as a contributor to the fluorescence of aged fingermark spot F/G. Anthranilic acid gave a yellow color reaction when developed with

Ehrlich's reagent. None of the fingermark spots resulted in a yellow spot after spraying with Ehrlich's reagent. No match was found between the excitation and emission spectra of anthranilic acid and the obtained spectra from the eluted fingermarks. Based on these findings, we excluded anthranilic acid as a major contributor to the autofluorescence of fingermarks.

N-Formylkynurenine is a blue fluorescent product from tryptophan oxidation [3]. The eluted formylkynurenine resulted in one elongated spot with an Rf-value of 0.46. Spot C obtained from fingermarks stored in a dark room cannot be excluded based on the Rf-value. However, spot C is not elongated and the fluorescent color is quite different than the fluorescent color of eluted formylkynurenine. The excitation and emission spectra of formylkynurenine did not result in a match with eluted fingermark spots. If formylkynurenine is present in aged fingermarks, its contribution is inferred to be minimal. Based on the fluorescent color, the elongated size of the fluorescent spot and the lack of a fluorescent spectral match, N-formylkynurenine was excluded of being a fluorescent fingermark component.

3-Hydroxyanthranilic acid is a blue fluorescent component [1-2]. The Rf-value of the eluted 3-hydroxyanthranilic acid is found to be close to the Rf-value of anthranilic acid, namely 0.96. Hydroxyanthranilic acid cannot be excluded as a fingermark component, since spot F/G showed a similar Rf-value and fluorescent color. No reaction with Ehrlich's reagent was observed, whereas spot F/G obtained from the fingermarks resulted in a pink/purple reaction with Ehrlich's reagent. No match was found between the excitation and emission spectra of hydroxyanthranilic acid and the obtained spectra from the eluted fingermark spots. Based on our findings, 3-hydroxyanthranilic acid was excluded as a fluorescent fingermark component.

3-Hydroxykyurenine displays a strong yellow fluorescence [1-2]. This tryptophan metabolite resulted in an elongated spot with an Rf-value of 0.48. No elongated spots were visible on the developed plates of the aged fingermarks. No spots with a similar Rf-value as the one obtained from 3-hydroxykynurenine could be found in the eluted fingermark spots. No spectral match could be obtained from hydroxykynurenine and spectra obtained from eluted fingermark spots. Based on these finding, 3-hydroxykynurenine was excluded as being a contributor to the autofluorescence of aged fingermarks.

3-Hydroxyquinaldic acid is a metabolite of kynurenic acid and thus tryptophan, and was eluted in a bright yellow fluorescent spot with an Rf-value of 0.91 [4], corresponding to the Rf-values of spot F/G. The fluorescent spot of kynurenic acid was of a different color than the blue fluorescent spot F/G. When developed with Erhlich's reagent a bright yellow color could be observed. None of the fingermark spots gave a bright yellow reaction when developed with Ehrlich. No match was found between the excitation and emission spectra of hydroxyquinaldic acid and the obtained spectra from the

eluted fingermarks. 3-Hydroxyquinaldic acid was therefore excluded as being a major contributor to the fluorescence of aged fingermarks.

Kynurenic acid is known as a yellowish green fluorescent tryptophan metabolite [2, 5] and eluted into three fluorescent spots, one yellowish spot with an Rf-value of 0.80, a brighter purple/blue spot (Rf-0.93) and a small yellowish fluorescent spot with an Rf-value of 0.98. None of these three spots can be excluded of contributing to the autofluorescence of aged fingermarks. Similarities of Rf-values and fluorescent colors were found with spot D-G (office conditions and dark). No coloration was observed when developed with Ehrlich's reagent. Spot D and E obtained from the aged fingermarks did also not give a color reaction with Ehrlich's reagent., whereas spot F/G gave a purple reaction with Ehrlich's reagent. Also, no spectral match could be obtained from kynurenic acid and spectra obtained from eluted fingermark spots. Based on these findings, kynurenic acid cannot be excluded of being one of the contributors to the autofluorescence of aged fingermarks. However, if present in aged fingermarks its contribution to the autofluorescence of fingermarks will be minimal.

Kynurenine is a metabolite of tryptophan and has a blue fluorescent color [1, 6]. Kynurenine is eluted into two different spots, one strong fluorescent elongated blue spot (0.56) and one weaker yellowish spot (Rf-value 0.93). In a previous study kynurenine was indicated as one of the contributors to the autofluorescence of fresh fingermarks [6]. Kynurenine could not be excluded as a fingermark aging component, based on the Rf-value and fluorescent color. The yellow fluorescent spot did not react with Ehrlich's reagent. The blue fluorescent spot reacted to produce a yellow spot, none of the spots eluted from fingermarks with similar Rf-value displayed this color reaction. No spectral match could be obtained from kynurenine and the eluted fingermark spots. If kynurenine is present in aged fingermark residues, its contribution to the autofluorescence is inferred to be minimal.

REFERENCES

1. Dalgliesh C: **The relation between pyridoxin and tryptophan metabolism, studied in the rat**. *Biochem J* 1952, **52**(1):3.
2. Mason M, Berg CP: **A chromatographic method for the detection of tryptophan metabolites.** *J Biol Chem* 1951, **188**(2):783-788.
3. Pirie A: **Fluorescence of N'-formylkynurenine and of protein exposed to sunlight.** *Biochem J* 1972, **128**(5):1365.
4. Takahashi H, Kaihara M, Price JM: **The conversion of kynurenic acid to quinaldic acid by humans and rats.** *J Biol Chem* 1956, **223**(2):705-708.
5. Satoh K, Price JM: **Fluorometric determination of kynurenic acid and xanthurenic acid in human urine**. *J Biol Chem* 1958, **230**(2):781-789.
6. Lambrechts SAG, van Dam A, de Vos J, van Weert A, Sijen T, Aalders MCG: **On the autofluorescence of fingermarks**. *Forensic Sci Int*2012, **222**(1–3):89-93.